Gluconeogenesis

Introduction

Gluconeogenesis can occur **only in hepatocytes**. In the glucagon-dominant state, when there is no dietary glucose coming into the system, tissues still use glucose as their major source of fuel. That glucose must come from somewhere. That somewhere is the liver. Be careful. The liver does use glucose in the insulin-dominant state to build ATP (just like all cells). It's "default glycolysis" just like every cell in the body. But the liver has additional coding that allows it to deny that default glycolysis, and instead go the other way. **Only the liver can build glucose**, and **only the liver can release glucose into the bloodstream**. We learned in Metabolism #3: *Glycolysis* that the K_M of glucokinase (liver) was 100 times that of hexokinase (muscle) (and so has 1/100th its affinity). The reason is that muscle only takes glucose and uses it. When glucose gets into the muscle cell, the cell traps it there so it can use it. The liver is designed for programmed-release glucose. All other cells are designed to hold onto glucose, regardless of the insulin-or-glucagon state.

This lesson offers a quick review of glycolysis, focusing only on the **irreversible reactions of glycolysis**. Then, we will talk about how default glycolysis is circumvented in the liver, the mechanisms the liver has developed to get around them. There are three reactions the liver has adapted to overcome the "default glycolysis" programming that all cells have. Only the liver has the programming to go the other way.

The reactions of gluconeogenesis are:

1. Pyruvate-in-the-mitochondria to PEP-in-the-cytoplasm using the three-step process of:
    a. Pyruvate carboxylase
    b. Malate shuttle
    c. PEP carboxykinase
2. Dephosphorylating fructose-1,6-bisphosphonate (reversing PFK-1) with **fructose-1, 6-bisphosphatase**
3. Release of phosphorylated glucose (reverse glucokinase) with **glucose-6-phosphatase**

Fed State (Insulin) vs. Fasting (Glucagon): Teleology

The liver can make glucose. In the well-fed body with plenty of glucose to go around, the liver will not need to make any glucose. When there is plenty of sugar around, the pancreas makes insulin. So, in the fed state, when glucose is plentiful from the meal, **insulin goes up**. Brain cells take up and burn glucose to make ATP. Adipose takes up glucose and burns it to ATP. Muscles burn glucose to ATP and store it as local glycogen. That's all those cells know how to do. Take up, use, and sometimes make glycogen for later. Even the liver, with all its specialties, in the **insulin-dominant state** does **the same default as all other cells**—burn glucose to ATP and store some as glycogen. The liver is cleverer than the muscle cells, though, and can do some pretty neat things with that glucose (like build fatty acids). But for the most part, in reference to carbohydrates, the liver in a fed state = insulin = glycolysis.

If that same body is fasting, or hours out from a meal, the mealtime glucose has been depleted. But the body doesn't die of hypoglycemia. The pancreas, seeing declining sugars, **turns down insulin**, and **turns glucagon up**. Now in a glucagon-dominant state most cells do the same thing they did in the insulin-dominant state: take up and burn glucose to ATP. Those tissues that have glycogen now use it. Adipose mobilizes fatty acids. But in regards to glucose, **nothing changes**. The liver, though, being the savior of the fast, **sees glucagon** and **switches to gluconeogenesis**, making glucose for the body to use.

Consider metabolism as being either insulin-dominant or glucagon-dominant. See the **liver** as being either **glycolysis on** (insulin-dominant state, therefore gluconeogenesis off) or **gluconeogenesis on** (glucagon-dominant state, therefore glycolysis off). If one is on, the other is off. Keep it binary.

And this fight—glucagon gluconeogenesis vs. insulin glycolysis—occurs only in the liver. Let's go through the reactions one at a time.

Step 1: The Hard Part—Reversing Pyruvate Kinase

During glycolysis, glucose becomes pyruvate in the cytoplasm, then goes as pyruvate to the mitochondria. In gluconeogenesis, pyruvate in the mitochondria will become glucose in the cytoplasm. **Gluconeogenesis starts with pyruvate in the mitochondria** and **ends with glucose in the cytoplasm**. Most of gluconeogenesis must be in the cytoplasm. In order to be the reverse of glycolysis there must be phosphorylations and dephosphorylations, and phosphorylation means "trapped in compartment." So if glucose is going to get into the blood, it can't be stuck in the mitochondria, so it must at least end in the cytoplasm.

Most of the reactions of glycolysis are reversible—gluconeogenesis uses the default pathway and adds only three processes to overcome the irreversible steps.

What Can't Happen 1: Pyruvate. Pyruvate-in-the-cytoplasm instantly becomes pyruvate-in-the-mitochondria, and since gluconeogenesis must occur in the cytoplasm, **gluconeogenesis cannot produce pyruvate in the cytoplasm**. That means, if we are to reverse glycolysis, we have to make a compound in the cytoplasm that is **1,6-bisphosphofructose** upstream-for-glycolysis (came before pyruvate).

What Can't Happen 2: Acetyl-CoA. Acetyl-CoA comes from two places: pyruvate dehydrogenase (pyruvate to acetyl-CoA) in the mitochondria, and fatty acid oxidation in the mitochondria. "In the mitochondria" is important because acetyl-CoA has one destiny—TCA/ETC to become ATP. **Acetyl-CoA can never become glucose**. But it can turn the TCA to produce energy, a product of fatty acid oxidation stimulated by glucagon.

What Must Happen: Pyruvate-in-the-mitochondria is the **only source of glucose** in gluconeogenesis. Pyruvate-in-the-mitochondria must be made into another compound, shuttled to the cytoplasm, and be phosphorylated to trap it there. That means that the **reversal of pyruvate kinase** (one step, glycolysis) requires **three sequential steps** (three steps, gluconeogenesis).

In that glucagon-dominant state, pyruvate is turned into **oxaloacetate** by **pyruvate carboxylase**. Oxaloacetate was what we combined acetyl-CoA with to start the Krebs cycle. But unlike in the fed state, we are not starting with acetyl-CoA. Therefore, the wheel doesn't turn. THIS oxaloacetate isn't needed for the Krebs cycle. Instead, using the **malate shuttle** the oxaloacetate in the mitochondria is turned to malate, shuttled to the cytoplasm, and transformed back into oxaloacetate. The malate shuttle effectively is a one-way portal from the mitochondria to the cytoplasm for oxaloacetate. On the cytoplasmic side, **PEP carboxykinase** turns oxaloacetate to PEP. Not pyruvate, PEP. We skipped over pyruvate.

Because I had a tendency to confuse the two names, I say "PEP see kay" (PEPCK) for PEP carboxykinase, and say pyruvate carboxylase in full.

A three-step process—pyruvate carboxylase (requires ATP), malate shuttle, and PEP carboxykinase (requires energy)—is required to get that pyruvate back to PEP. ONE step going from PEP to pyruvate during glycolysis, THREE different steps to get to PEP. If we keep with the "add-on-to-the-default" concept from glycolysis, it makes sense that the liver, the only organ that can make glucose, the only organ that can circulate glucose for all other organs to use, developed a complicated and roundabout method for doing it.

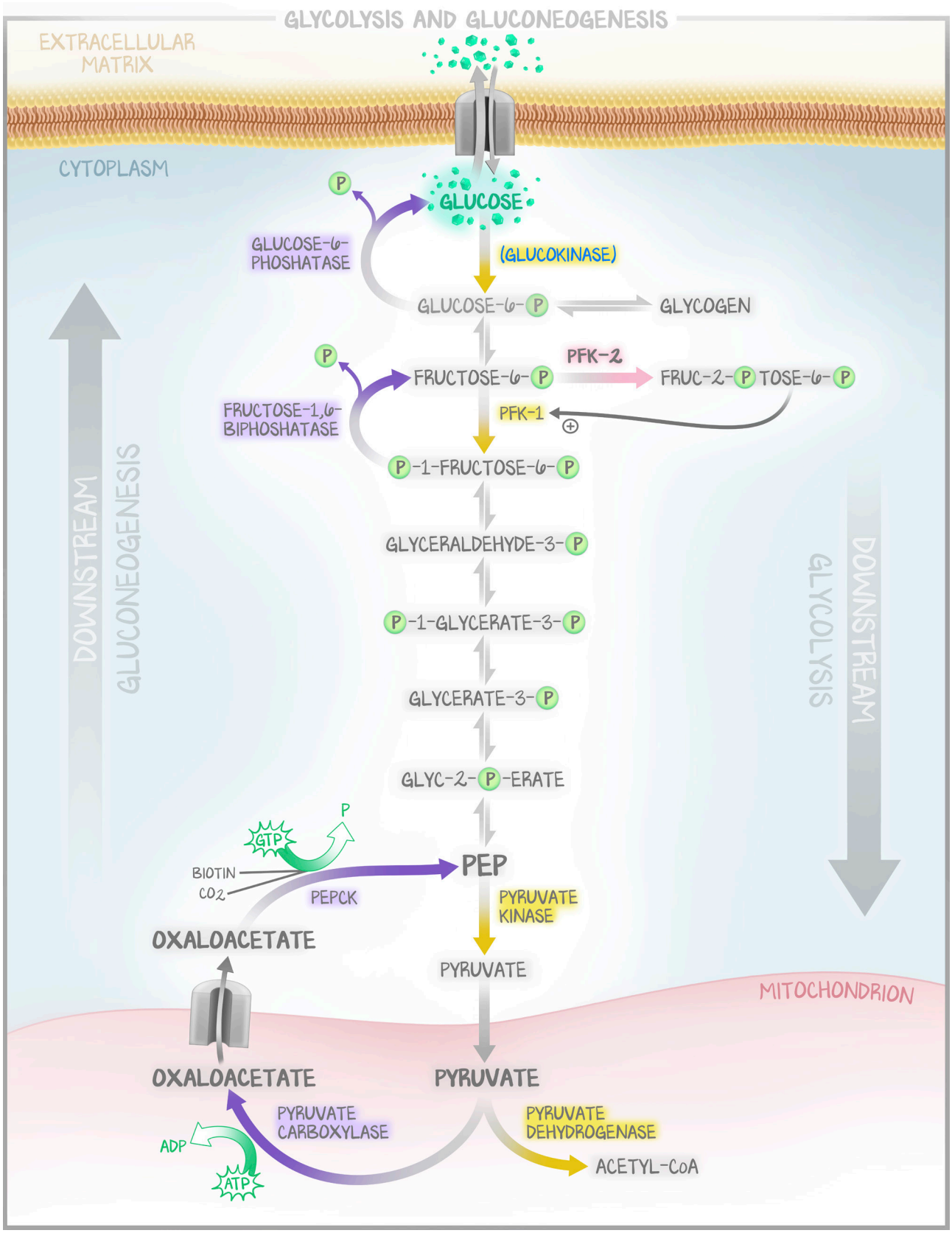

Figure 8.1: Complete Gluconeogenesis
You do not have to memorize all the steps—only those that manage to get around glycolysis-irreversible steps.

Step 2: The Easy Part—Reversing PFK-1; and Step 3: Reversing Glucokinase

From PEP, glycolysis just goes backwards. To ensure that gluconeogenesis is favored over glycolysis, substrates that are downstream-for-gluconeogenesis must be removed, favoring their formation in the direction of gluconeogenesis. Coincidentally (and conveniently), to reduce favorability of glycolysis, we should remove substrates that are upstream-for-glycolysis. What is downstream-for-gluconeogenesis is upstream-for-glycolysis. We want to remove the substrate downstream-for-gluconeogenesis, and "removal" is accomplished with irreversible reactions. The **irreversible steps of glycolysis** are also the **irreversible steps of gluconeogenesis**. Gluconeogenesis must have a mechanism for reversing PFK-1 and glucokinase AND ensuring regulation that turns on gluconeogenesis and turns down glycolysis.

PFK-1 takes fructose-6-phosphate to fructose-1,6-bisphophate. We just need to get that phosphate off. Kinases put phosphates on, phosphatases remove them. **Fructose-1,6-bisphosphatase** is a different enzyme than PFK-1, doing a different reaction than PFK-1, but gets us to where we want to be.

Glucokinase traps glucose in the cell. It's a kinase, adding a phosphate. To undo it, following the PFK-1 example, we'd need a phosphatase. **Glucose-6-phosphatase** removes the phosphate and makes glucose. Glucose can leave the liver cell. Glucokinase (the glucose-trapping enzyme of the liver) has a very low affinity for glucose; it's really poor at trapping it. Good thing, since we want to release it. Once released into the blood, that glucose goes to other cells. Glucose in the cytoplasm of other cells is readily trapped there by hexokinase (the glucose-trapping kinase of other cells). The liver releases glucose while all the other cells take the glucose the liver makes.

Regulation

This is obviously going to be an endeavor, being **so different** than the default (glycolysis only), and **so important** (without gluconeogenesis, the organism does not live). We are going to review all levels of regulation in the next lesson. But highlighting key enzymes in gluconeogenesis before we leave is necessary.

Pyruvate carboxylase is stimulated by acetyl-CoA (which also inhibits pyruvate dehydrogenase), pushing any pyruvate that makes it to the mitochondria towards gluconeogenesis. **Substrate-level regulation**.

Pyruvate dehydrogenase is inhibited by acetyl-CoA, ensuring that during fatty acid catabolism, no more pyruvate will be wasted on TCA-ETC, and will instead be used for gluconeogenesis. **Only in the liver** does insulin stimulate pyruvate dehydrogenase. The lack of insulin during the glucagon-dominant state results in less insulin, and even less pyruvate dehydrogenase activity.

PEP carboxykinase (PEPCK) is **induced by glucagon**. Induction means **increased gene expression**. In the fasting state, insulin decreases, glucagon increases, and the expression of the liver changes to favor gluconeogenesis.

Fructose-1,6-bisphosphatase (or "FBPase") does the opposite of PFK-1, and also has the exact opposite response to regulation by PFK-1. That regulation is mediated by the substrate fructose-2,6-bisphosphate (Fru-2,6-P_2). Fru-2,6-P_2 stimulates PFK-1 and **inhibits FBPase**. Fru-2,6-P_2 comes from PFK-2. Technically, ATP stimulates FBPase and AMP inhibits it (substrate-level), but focus on the PFK-2 part of regulation.

FBPase-insulin: Insulin dephosphorylates and therefore activates PFK-2. Under insulin, Fru-2,6-P_2 is made. Under insulin, Fru-2,6-P_2 inhibits FBPase and stimulates PFK-2.

FBPase-glucagon: **Glucagon phosphorylates** and therefore **deactivates PFK-2**. Under glucagon, **less Fru-2,6-P_2 is made**. Under glucagon, Fru-2,6-P_2 does **not inhibit FBPase** and does **not stimulate PFK-1**.

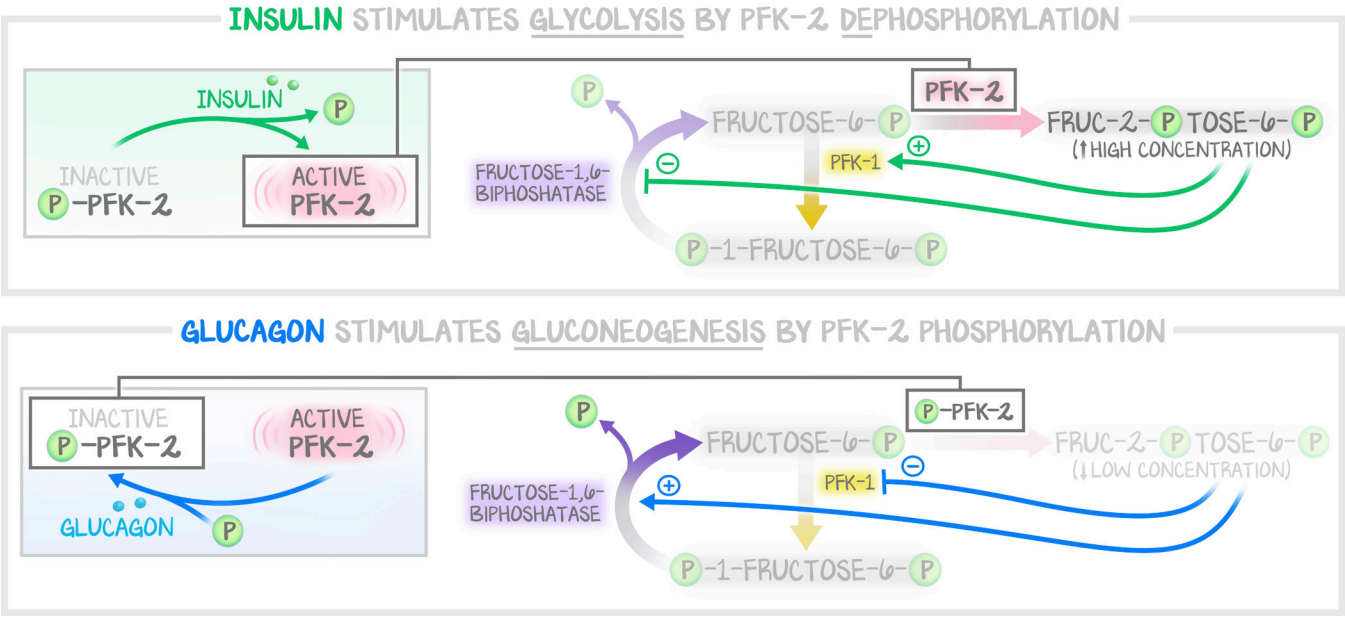

Figure 8.2: PFK-2 Regulation by Glucagon and Insulin
It is through direct competition over the phosphorylated state of PFK-2 that glucagon and insulin indirectly regulate gluconeogenesis and glycolysis.

Glucokinase is induced by insulin. In a glucagon-dominant state, there is reduced gene expression for glucokinase, an enzyme that already has a low affinity for glucose. Low affinity, less enzyme, very little glucose will be trapped.

To be clear: there is gene expression activation (↑ glucagon = ↑ PEPCK) and gene expression inhibition (↓ insulin = ↓ glucokinase); there are hormone-induced phosphorylation states (↑ glucagon = ↑ phosphorylation of PFK-2 = ↓ activity; ↓ insulin = ↓ dephosphorylation of PDH = ↓ activity) and substrate-level regional control (acetyl-CoA on pyruvate carboxylase, ATP on fructose-1,6-bisphosphatase).

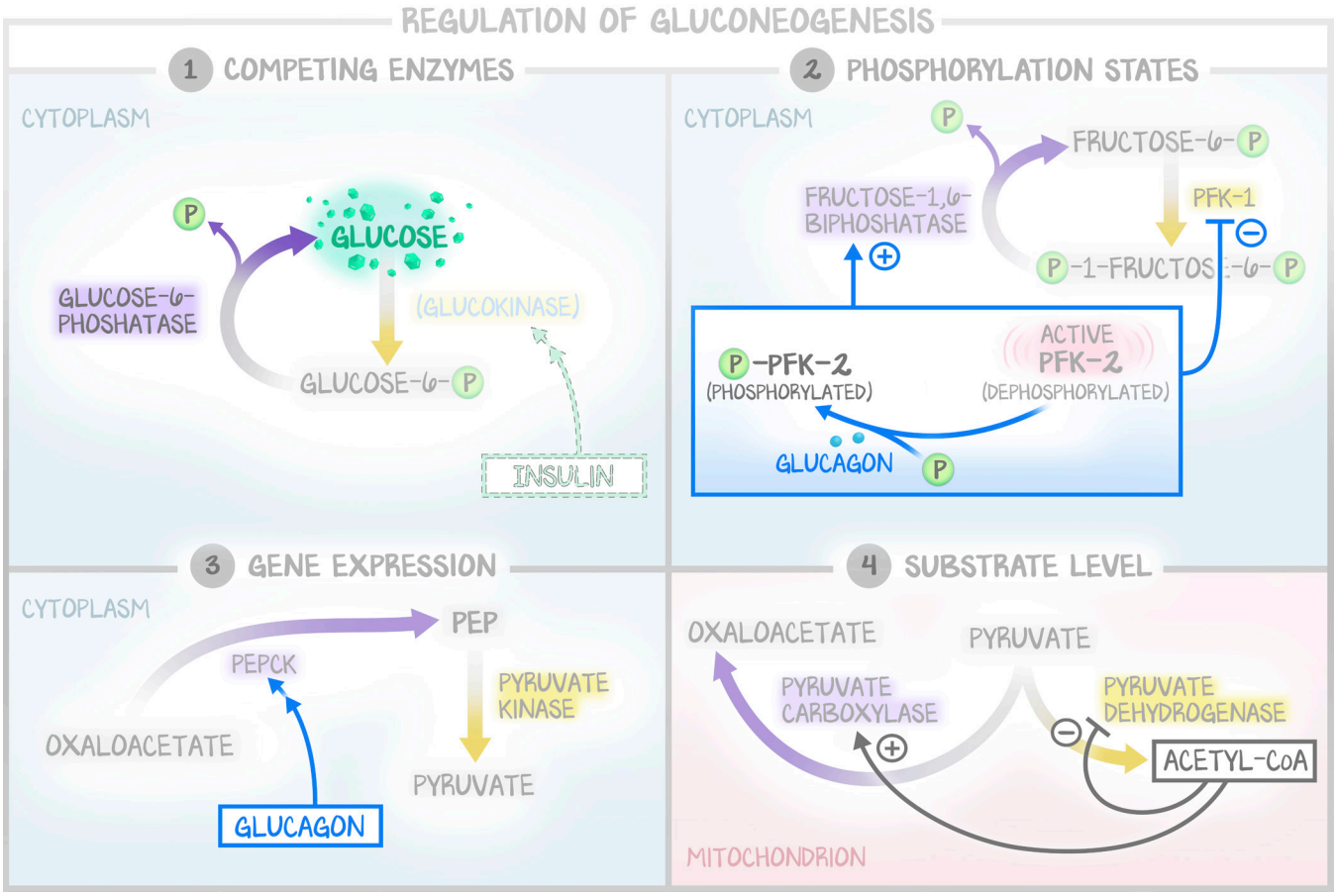

Figure 8.3: Regulation of Gluconeogenesis
Much is involved in the regulation of gluconeogenesis that combines substrate-level regulation, hormonal balance, and gene expression.